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Genetic Linkage and Association Analysis for Loneliness in Dutch Twin and Sibling Pairs Points to a Region on Chromosome 12q23–24

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We obtained evidence from a large study in Dutch twins ($N = 8387$) and siblings ($N = 2295$) that variation in loneliness has a genetic component. The heritability estimate for loneliness, which was assessed as an ordinal trait, was 40% and did not differ between males and females. There were 682 sibling pairs with genotypic (around 400 microsatellite markers) data. We combined phenotypic and genotypic data to carry out a genome scan to localize QTLs for loneliness. One region on chromosome 12q23.3–24.3, showed near suggestive linkage. Genetic association tests within this region revealed significant association (p-value 0.009) with one of the alleles of marker D12S79 and with one of the alleles of neighbouring marker D12S395 (p-value 0.043). We review evidence for linkage in this region for psychiatric disorders and discuss our findings within this context.

KEY WORDS: Association; heritability; linkage; loneliness; ordinal data; QTL; sib-pair.

INTRODUCTION

Loneliness has been described by Weiss (1973) as "a gnawing ... chronic disease without redeeming features" which may occur when one's intimate and social needs are not adequately met (Baumeister and Leary, 1995). The core experience of loneliness consists of social isolation and the absence of both relational and collective connectedness (Hawkley *et al*., 2005; Russell *et al*., 1980). There is now substantial evidence that loneliness is at the heart of a constellation of socio-emotional states, which include self-esteem, mood, anxiety, anger, optimism, fear of negative evaluation, shyness, social skills, social support, dysphoria, and sociability (see reviews by Duck *et al*., 1994; Ernst and Cacioppo, 1999; Peplau and Perlman, 1982; Berscheid and Reis, 1998; Rook, 1988; Shaver and Brennan, 1991).

Recently, attention has been paid to how genetic and environmental factors influence the development of individual differences in loneliness. McGuire and Clifford (2000) examined the heritability of loneliness in children and found significant genetic ($h^2 = 55\%$ and 48%, respectively) contributions to individual differences in loneliness. To examine the genetic contribution to variation in loneliness in adults, Boomsma *et al*. (2005) conducted a study based on data of 8387 adult twins from the Netherlands Twin Register. It was found that individual differences in loneliness demonstrated considerable temporal stability and heritability. The estimate of heritability was 48%, and all environmental influences were unique to each individual. The genetic contributions to adult loneliness were similar in males and females and in a younger and an older cohort. Finally, no qualitative sex differences in heritability were found, indicating that the same genes influence loneliness in both sexes.

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The heritability of loneliness is comparable to that of other complex traits which have been the subject of genome scans to identify susceptibility loci. As is the case for other complex traits, heritability may be caused by polymorphisms in several genes. Because a large number of genetic and environmental factors may contribute to the liability of loneliness, isolating the genetic component is a daunting undertaking (Stenger *et al*., 2005). We collected genotypic marker data and phenotypic information in 682 sibling pairs and their parents and aim, through linkage and association analysis, to elucidate candidate regions which may harbor genes influencing variation in loneliness. Loneliness was analyzed as an ordinal phenotype, using a threshold model which assumes a latent normally distributed liability (Falconer, 1989; Lynch and Walsh, 1998). The ordinal phenotype and the continuous liability are linked through thresholds and the liability was treated as an unobservable quantitative trait (Yi *et al*. 2004).

METHODS

Participants and Phenotypic Information

In 1991 the Netherlands Twin Register (NTR) started a longitudinal survey study of health and lifestyle in adolescent and adult twins and their family members (Boomsma *et al*., 2002). Surveys were mailed out in 1991, 1993, 1995, 1997, 2000 and 2002/3. Twins were asked to participate in all waves, siblings were included since 1995. Five surveys contained items on loneliness from the YASR (Achenbach, 1990), which could be answered on a 3-point scale. Based on factor analyses, we selected 2 items (item 12: I feel lonely, and item 33: Nobody loves me). Information on loneliness was quantified by averaging over items and over years of observation (Boomsma *et al*., 2005). There were phenotypic data for 8387 twins, and for 2295 full siblings.

DNA Collection and Genotype Data

Genotype data came from 2 studies: a cardiovascular study of unselected DZ twin pairs (Heijmans *et al*., 2005) and a study of DZ twin and sibling pairs (Boomsma *et al*., 2000) who were selected for anxious depression. In the cardiovascular study, DNA was collected from blood samples. For 234 individuals, there were phenotypic data on loneliness. Genotyping in these samples was carried out by the Dept of Molecular Epidemiology (Leiden). A 419 marker genome scan (8.34 cM spacing) was done using a combination of in-house markers (ALFexpress automated sequencer (Amersham Pharmacia Biotech)) as described by Beekman *et al*. (2003), markers from the Weber screening set 8 (ALFexpress) and from the Human Linkage Set v2.5 MD10 and HD5 (ABI Prism DNA Analyzer 3700 (Applied Biosystems)).

DNA from buccal swabs was collected as part of the NETSAD project (Boomsma *et al*., 2000) in sibling pairs selected for anxious depression, with additional, non-extreme siblings in the family included in the DNA collection. For 523 siblings (50 of which were also genotyped as part of the cardiovascular study) phenotypic loneliness data were available. Genotyping in the NETSAD samples was performed by the Mammalian genotyping service (Marshfield, USA). A 379 autosomal marker genome scan (9.44 cM spacing) was done using microsatellite screening set 10 (Yuan *et al*. 1997) with few alternative markers. Excessive recombination rates were observed for five markers indicating potential problems. Those markers were not included in the analysis (D1S468; D1S1627; D11S1985; D11S2006 and D20S159-UT1307). Five other markers are currently inconsistently mapped and were also excluded. There were 100 markers typed in both scans. Mendelian errors were detected using PEDSTATS and unlikely double recombinants using MERLIN; both types of error were removed using PEDWIPE (Abecasis *et al*., 2002). Pedigree relationships were checked with GRR (Abecasis *et al*., 2001). Marker location was taken from an integrated genetic map with interpolated genetic map positions (http://www2.qimr.edu.au/davidD/). The position is in Decode cM (Kong *et al*., 2002), estimated via locally weighted linear regression from the Build 34.3 (and 35.1) physical map positions and published Decode and Marshfield genetic map positions. Parents were typed for between 185 and 375 markers (mean = 352, SD = 33). For offspring, the number of typed markers ranged from 184 to 678, with an average of 378 (SD = 78). Genotypic data as well as data on loneliness were available for 707 offspring, from 277 families (for 102 families both parents were genotyped and for 40 families one parent was typed), forming 682 sib pairs.

QTL Analysis

The phenotypic data were analyzed with a threshold model, with 3 thresholds dividing the liability to loneliness into 4 categories, and different thresholds for males and females (Boomsma *et al*.,

2005). Because the genotyped pairs are not a random sample, thresholds were fixed at the estimates from the model in which data from all twins and siblings were analyzed. Variation in liability to loneliness was decomposed into variation due to a QTL (σ^2_q), additive polygenic influences (σ^2_a), and non-shared environmental influences (σ^2_e), using structural equation modelling as implemented in Mx (Neale *et al*., 2003). Estimates of the variance component associated with a putative QTL were obtained by using a $\hat{\pi}$ approach, in which the covariance due to the QTL for a sib-pair is modelled as a function of the estimated proportion of alleles shared identical by descent. The variance–covariance matrix for pair *j*,*k* of the *i*-th family (Ω_{ijk}) is given by:

$$\Omega_{ijk} = \begin{array}{ll} \sigma^2_a + \sigma^2_q + \sigma^2_e & \text{if } j = k \\ \rho\sigma^2_a + \hat{\pi}_{ijk}\sigma^2_q & \text{if } j \neq k \end{array}$$

σ^2_a, σ^2_q and $c\sigma^2_e$ denote the background and the QTL additive genetic and environmental variances. For DZ twin and sib pairs $\rho = 1/2$ (twice the kinship coefficient). For DZ and sibling pairs $\hat{\pi}$ depends on the IBD status of the pair and is obtained as: $\hat{\pi} = 0.5p_{(\mathrm{IBD}=1)} + p_{(\mathrm{IBD}=2)}$, where $p_{(\mathrm{IBD}=1)}$ denotes the probability that the pair is IBD = 1 and $p_{(\mathrm{IBD}=2)}$ denotes the probability that the pair is IBD = 2. Estimates for IBD probabilities were obtained from Merlin (Abecasis *et al*. 2002). Significance of variation of the QTL was evaluated by the likelihood ratio test, from which the LOD score was calculated (Sham, 1998) by dividing the test statistic χ^2 by 2 ln 10 (~ 4.6).

Permutation Tests

To obtain empirical estimates of genome-wide significance levels, 1000 permutations of the dataset were performed, keeping both the family structure and the IBD structure intact. These permutations account for uneven marker spacing and informativeness (Churchill and Doerge, 1994). Permuted datasets were obtained as follows: Each row in the observed dataset represents one family and contains phenotypes and IBD probabilities across the whole genome for all pairs within that family. This file is split into a phenotype file and an IBD file. Families are labeled with unique numbers one through *n*. The phenotypic data are then shuffled by taking a random permutation of the indices 1, ... , *n* and matching the *i*th phenotypic trait value to the family with index given by the *i*th element of permuted indices. This permuted vector of traits is matched with the original (non-permuted) genotypic information for all families. One thousand permuted datasets were generated; each permuted dataset was then analyzed. The threshold for suggestive linkage was calculated by recording the maximum LOD-score for each chromosome in 1000 runs, and determining what LOD-score occurs a 1000 times out of 22,000. This represents the average maximum peak size expected once per genome scan (Nyholt *et al*., 2005). The threshold for suggestive linkage for the ordinal trait loneliness was 1.58. The significant linkage threshold obtained by determining the maximum LOD-score for each scan, and was then defined as the LOD-score occurring in 50 of the 1000 permutations corresponding to a probability of 0.05 in a genome scan (Churchill and Doerge, 1994; Lander and Kruglyak, 1995). The threshold for significant linkage was 2.87.

Genetic Association Testing

Genetic association tests were conducted with the extended TDT test proposed by Monks and Kaplan (2000) and implemented in the program QTDT (Abecasis *et al*., 2000). This test uses a weighing scheme that provides a conservative test of association in families with multiple offspring, and uses parental genotypes if available. Because the program does not handle ordinal data with >2 categories, the ordinal variable was dichotomized to obtain an affection status by coding all individual with ordinal category 0 (i.e. always replied "no" to the 2 loneliness items) as unaffected, and all individuals with ordinal categories >0 as affected.

RESULTS

The previous study of loneliness included data from twins only. We repeated the heritability analyses of loneliness for twins ($N = 8387$) and their siblings. A maximum of two brothers and two sisters was added to the data set (data from additional brothers ($N = 33$) and sisters ($N = 66$) were not used). This added a total of 1019 brothers and 1276 sisters to the analyses. The heritability was estimated at 40% (95% CI = 0.35–0.44). The prevalence for males and females in the sample that is available for linkage and in the total samples is given in Table I.

Figure 1 shows the results of the whole genome scan. One region, 12q23.3–24.3, showed a LOD-score (1.38) just below the empirical threshold for suggestive linkage. The drop 1 LOD-score borders are

Table I. Prevalence of Loneliness Categories in the Sample Available for the Linkage Scan and in the Full Sample

	Loneliness category	Frequency	Percent	% Full sample
Females	0.00	214	52.3	49.7
	1.00	130	31.8	35.6
	2.00	58	14.2	11.9
	3.00	7	1.7	2.8
	Total	409		
Males	0.00	199	66.8	66.2
	1.00	69	23.2	25.3
	2.00	24	8.1	7.2
	3.00	6	2.0	1.3
	Total	298		

defined by markers D12S78 and D12S2078, which are 37 cM apart (see Figure 2).

We performed genetic association tests for loneliness' affection status with microsatellite markers within the 12q23.3–24.3 region. These markers included D12S1300 and D12S2078, and all markers in between, as indicated in Figure 2 ($N = 9$ markers; all markers were in Hardy–Weinberg equilibrium). We obtained a significant association between the marker allele with repeat length 169 of marker D12S79 (p-value 0.0094), and suggestive association within the same marker and allele with repeat length 157 (p-value 0.0656). For neighbouring marker D12S395 association was seen with the 235 repeat allele (p-value 0.0432), and suggestive association within the same marker and allele with repeat length 227 (p-value 0.0662). For the marker allele with repeat length 169 of marker D12S79 we observed that 54% of carriers of at least one allele was affected (i.e. scored non-zero on the loneliness trait), whereas 38% of subjects without the 169 allele was affected.

DISCUSSION

A genome-wide scan for loneliness was conducted by treating loneliness as an ordinal phenotype, using a threshold model assuming a latent continuous liability. Considering the moderate heritability of loneliness and the general reduced power of ordinal data versus continuous data the odds of finding significant linkage seemed rather low at the outset. However, the Lander and Kruglyak (1995) guidelines for suggestive (2.2) and significant (3.6) linkage apply to quantitative traits, and may be too stringent for ordinal traits. Permutation testing confirmed that the thresholds for suggestive and significant linkage were considerably lower, i.e. 1.58 and 2.87. We obtained one near suggestive result from a genome-wide linkage scan for loneliness for a region on chromosome 12q23–24. Genetic association tests with markers within this area showed significant association to marker D12S79 and to neighbouring marker D12S395, offering support for the linkage result.

This region on chromosome 12 has been implicated before in studies of psychiatric disorders. Table II summarizes the evidence for linkage in this region from studies of psychiatric disease, including bipolar disorder, major depression, schizophrenia and indices of anxiety, neuroticism and alcohol abuse (see also Craddock *et al.*, 2005; Hamet and Tremblay, 2005). This region on chromosome 12q was first implied at the chromosomes 12 and 16 workshop (Detera-Wadleigh, 1999) as a region with compelling evidence for a bipolar disorder susceptibility locus. This suggestion was further strengthened by the finding that Darier's disease, which maps to this region, co-segregates with bipolar disorder. However, there is strong evidence against the Darier causing mutation itself being the susceptibility risk factor for bipolar disease (Jones *et al.*, 2002). Neither was any evidence found for the DUSP6 gene on chromosome 12q23 (Toyota *et al.*, 2000). Shink *et al.*, (2005a, b) investigated the 12q24.31 region by saturating a 7.7 Mb genomic region with 20 microsatellite markers and obtained linkage support for bipolar disorder in this area. They next analyzed 32 genes for polymorphisms in coding sequences and intron/exon junctions. No strong support for any of the genes was found. A positive result was obtained recently with a haplotype in the gene encoding a transcription factor (regulatory factor X4: RFX4) on 12q23 by Glaser *et al.* (2005a). Their haplotype-bipolar association was supported by an association with microsatellite D12S2072. This marker, which was not included in our genome scan, is located between D12S78 and D12S2070/79. The same group (Glaser *et al.*, 2005b) also observed association in this region with Cux2, which is a potential regulator of neural cell adhesion molecule expression, and with hypothetical protein FLJ32356; but not with PAH (Green *et al.*, 2003), which we also investigated and for which we found no association.

The region on 12q23–q24 may contain a general vulnerability locus for psychiatric disorders. This possibility is reinforced by the finding of linkage for Neuroticism in this region. The 12q23–q24 region was, however, not reported in meta-analyses of bipolar disorder (Segurado *et al.*, 2003) or schizophrenia (Lewis *et al.*, 2003). Since the publication of

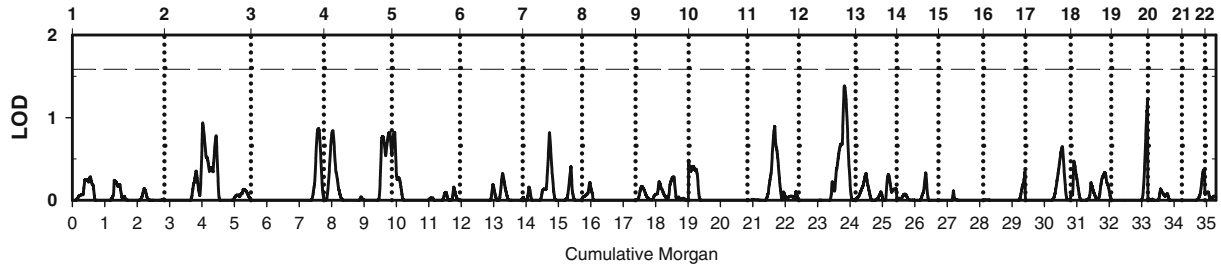

Fig. 1. Multipoint variance-component linkage of the 22 autosomes in 682 sib-pairs for loneliness. The X-axis plots genetic distance (in cM Haldane), and the Y-axis represents the LOD-score.

the meta-analyses several further genome scans were published (see Table II) with at least 2 genome scans reporting genome wide significance (Ewald *et al*., 2002; Shink *et al*., 2005a, b) within the 12q23–24 region.

Understanding the origins of variation in loneliness may be important for clinical as well as scientific purposes. Loneliness is associated with psychiatric and behavioral problems, including bipolar disorder, depression (Eisemann, 1984; 1985; Segrin, 1998), alcoholism (Akerlind and Hornquist, 1992; Bell, 1956), impaired sleep (Cacioppo *et al*., 2002a, b), and suicide (Goldsmith *et al*., 2002; Wenz, 1977). Patients with these disorders are socially isolated and have difficulties in maintaining friendships and relations. Loneliness may be a consequence as well as a

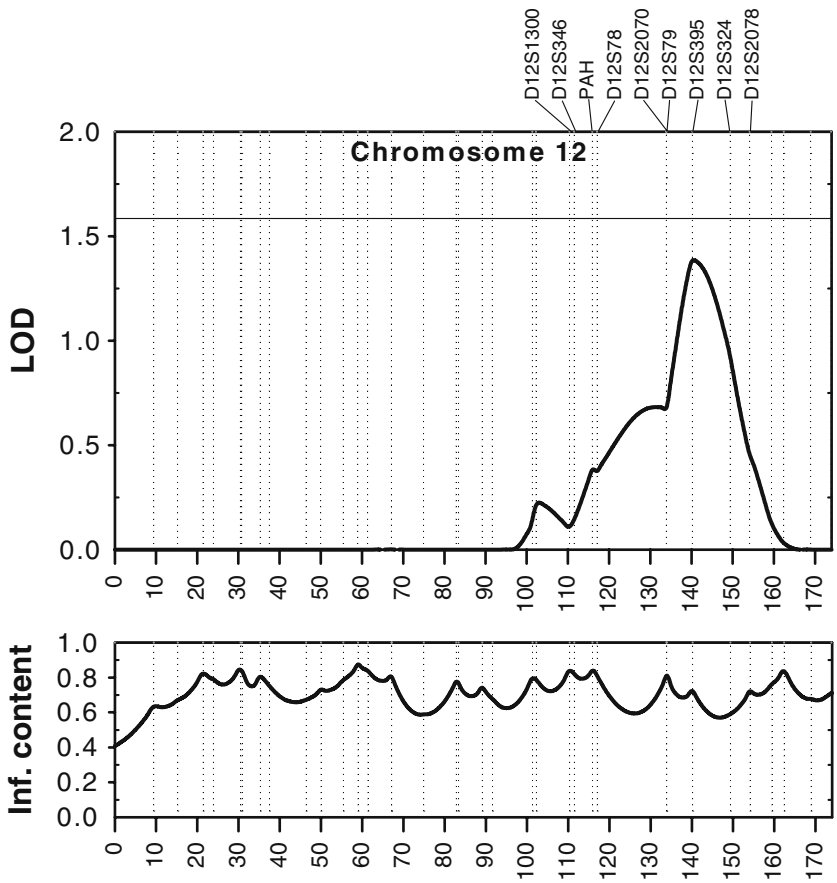

Fig. 2. Best evidence of linkage to loneliness on chromosome 12. The X-axis plots genetic distance (in cM Haldane), and the Y-axis the LOD-score. Markers are arrayed in map order along the top.

Table II. Overview of Linkage Findings for Chromosome 12q23–24

Study	Sample	N/Phenotype	Region
Green *et al.*, 2005	2 pedigrees UK; 45 markers in region of interest	Co-segregation of BPD and Darier's disease	12q23–24 (LOD = 4.77 between D12S1127 and D12S1646)
Curtis *et al.*, 2003	146 interviewed Ss from 2 UK and 5 Iceland families; 365 markers	39 Ss bipolar and 29 additional Ss unipolar	LOD = 2.8 at D12S342
Nash *et al.*, 2004	Selected sibling pairs a sample of 34,371 Ss UK; 408 marker genome-wide scan	711 Ss from 283 sibships selected on composite index G (e.g. anxiety, neuroticism)	LOD = 1.8 in 254 sister pairs (between markers D12S326 and D12S351) for G
Fullerton *et al.*, 2003	Selected sibling pairs from 20,427 independent sibships UK; 388 marker genome-wide scan	182 discordant, 205 low–low, 174 high–high sib pairs for Neuroticism	At 105 cM (−log *P* value = 4.74 at D12S346)
Shink *et al.*, 2005a	485 (101 new) Ss/41 families from SLSJ area in Quebec; 20 microsat. marker scan in candidate region on chromosome 12	105 BPI / SczBP, 42 BPII, 54 RUMD 57 UMD	12q24.31: association with markers in this region
Shink *et al.*, 2005b	394 Ss/20 families (18 new) from SLSJ area in Quebec; 380 marker genome-wide scan	77 BPI , 28 BPII, 43 RUMD, 45 UMD, 21 AD, 34 ADA	12q21.2–12q24.31 (LOD = 3.35 at D12S378)
Morissette *et al.*, 1999	114 Ss/1 large pedigree from SLSJ area; 332 markers. replication 34 Ss/1 pedigree; 18 chromosome 12 markers	44 BPI, 3 SczBP, 6 BPII, 11 RUMD, 18 UMD	12q23–q24
Maziade *et al.*, 2005	480 Ss/21 families Eastern Quebec; 350 marker genome-wide scan; 257 markers for follow-up	81 broad SZ, 72 broad BP	For BP: 12q23.1 (Z = 3.53 at D12S1030)
Camp *et al.*, 2005	718 affected Ss/87 extended pedigrees Utah; 629 marker genome-wide scan	516 RUMD, 714 RUMD or AD, 99 RUMD and AD	For RUMD and AD at 89.6 cM (LOD = 1.37 at D12S1667)
Abkevich *et al.*, 2003	1890 Ss from 110 pedigrees Utah; 629 microsat. marker genome-wide scan; 33 markers for follow-up	162 BPD (62 M), 784 RUMD (238 M), 161 UMD (62 Male)	For depression in males 12q23.1 (HLOD = 4.6 at D12S1030; HLOD = 6.1 at D12S1706 at follow-up)
Zubenko *et al.*, 2003	835 Ss/81 families USA; 520 Ss genotyped for 389 marker genome-wide scan	RUMD, (major) mood disorder, depression spectrum disorder	12q23.1 (LOD = 1.9 at D12S393)

Wilcox *et al.*, 2002	136 Ss/51 families with SZ/Scz probands USA; 459 marker genome-wide scan	Quantitative assessment of Negative, Positive and disorganized symptoms	At 104 cM (LOD = 2.97 at D12S1300; LOD = 2.12 at 109.5 cM (PAH gene)
Ekholm *et al.*, 2003	41 families Finland; 389 markers; additional markers for follow-up	101 BPI and Scz 36 BPI, BPII, Scz, BPD-NOS and RUMD	12q23 (Zmax = 2.0 at PAH gene at 109.5 cM)
Bailer *et al.*, 2002	86 Ss/8 families. Austria; 388 markers	5 SZ and 3 BPD families	At 142.2 cM (NPL = 2.43 / LOD = 0.97 at D12S97)
Ewald *et al.*, 2002	Two extended pedigrees Denmark; 613 markers; additional markers for follow-up	BPD, mania, Scz	At 148 cM (LOD = 3.42 at D12S1639)
Degn *et al.*, 2001	14 patients isolate Faroe Islands; 17 markers in candidate region	BPI and BPa	Single marker association at 145 cM (D12S342)
Brzustowicz *et al.*, 2000	288 Ss/22 families Canada (22 Celtic, 1 German); 381 markers	On average 3.6 Ss per family with SZ and Scz	109 cM (Zmax = 2.60 at PAH gene)

SLSJ: Saguenay-Lac-St-Jean area
AD: anxiety disorder
ADA: alcohol/drug abuse
BPD: bipolar disorder
BPa: bipolar affective disorder
BPD-NOS: Bipolar disorder-not otherwise specified
BPI: bipolar I
BPII: bipoar II
RUMD: recurrent unipolar major depression
Scz: schizoaffective disorder
SczBP: schizoaffective disorder, bipolar type
SZ: schizophrenia
UMD: single unipolar depression
Broad SZ: schizophrenia, schizophreniform and schizotypal personality disorder
Broad BP: BPI, BPII and RUMD
PAH: phenylalaline hydroxylase
HLOD: heterogeneity LOD score
LOD: logarithm of likelihood of linkage
NPL: nonparametric multipoint linkage score

predictor of psychiatric problems. Heikkinen and Kauppinen (2004) reported that loneliness predicted changes in depressive symptoms in a 10-year study of elderly Finns and Cacioppo *et al*. (in press) found that loneliness predicted subsequent changes in depressive symptoms and that there was evidence for reciprocal influences over time. The results of the current study suggest that alleles in LD with an allele at microsatellite marker D12S79 and at neighbouring marker D12S395 may contribute to individual differences in loneliness. Although additional research is required to determine their common phenotypic expression, it is possible that genes in this region may be involved in both affective and social regulation and dysregulation.

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